

106790" 27E48860

Patent No.: IBIS-0369
Title: MASS SPECTROMETRIC METHODS FOR
BIOMOLECULAR SCREENING
Inventors: Stanley T. Crooke, Richard Griffey and
Steven Hofstadler
Atty: Paul K. Legaard - Telephone: 215 568 3100
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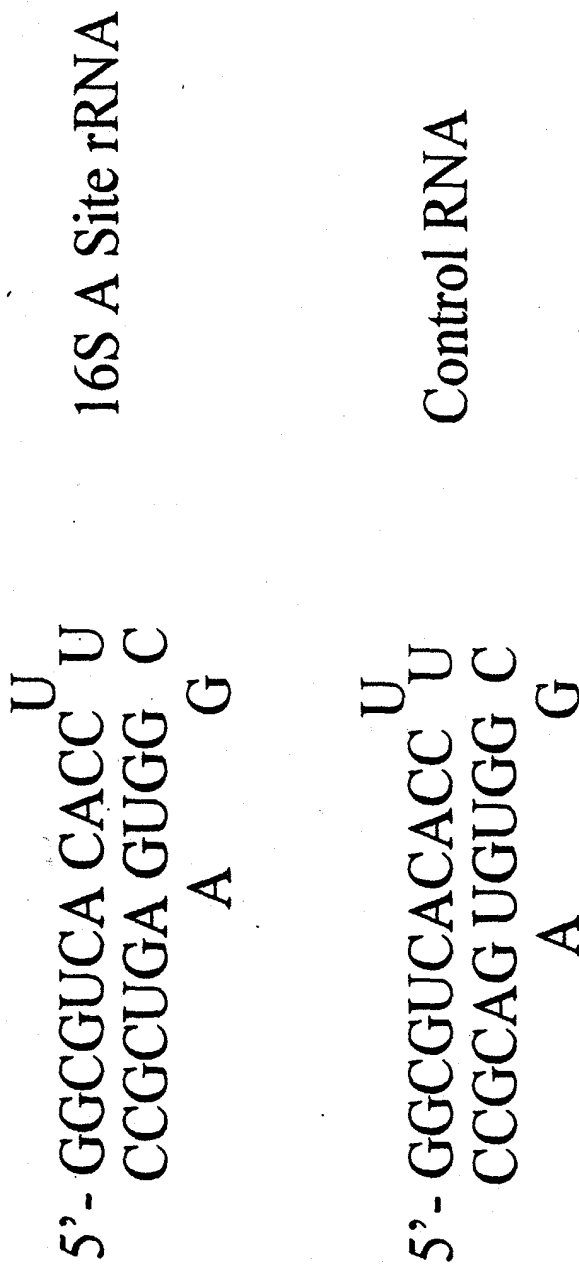


Figure 1. Sequence and structure of 27mer RNA target

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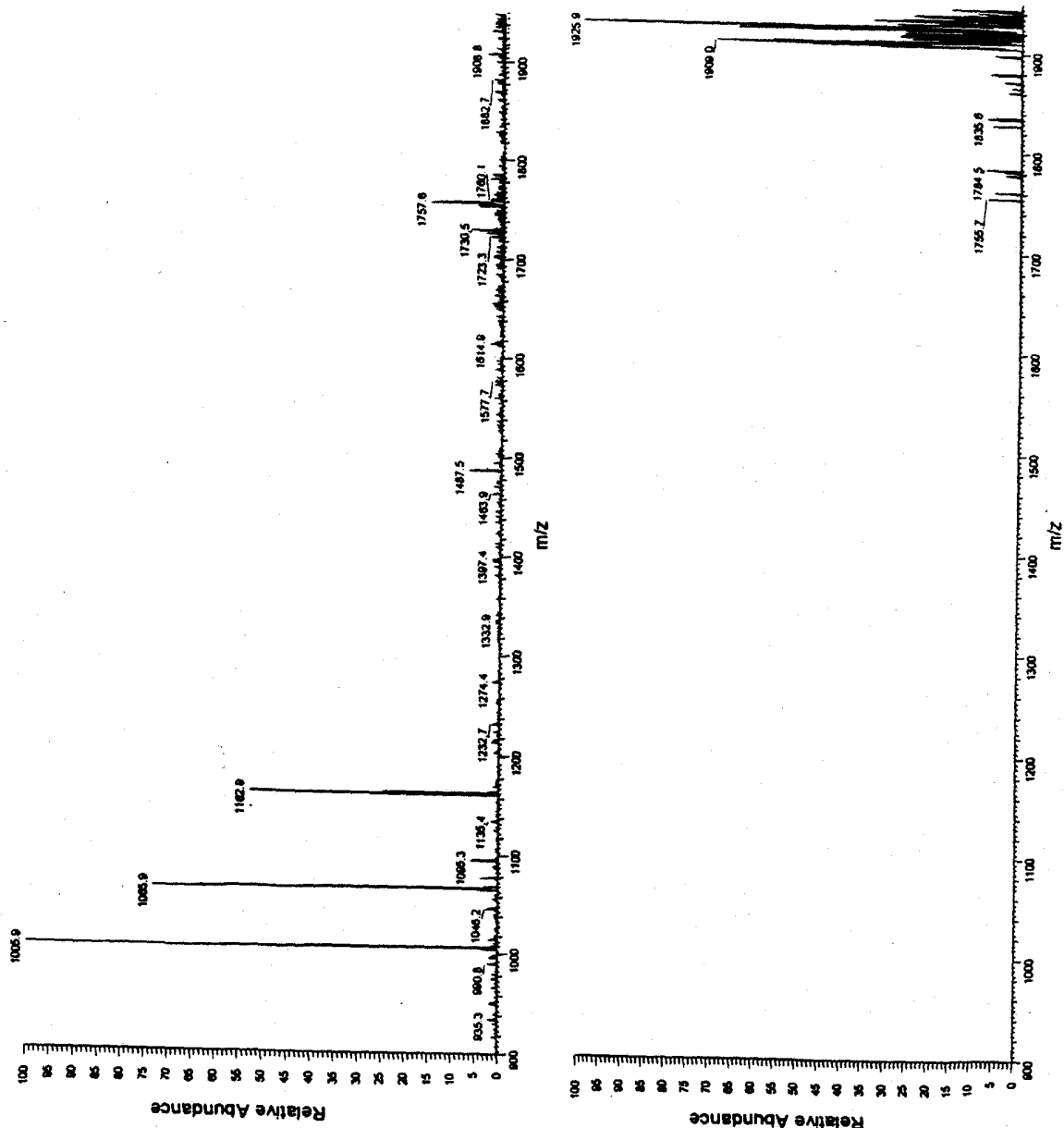


Figure 2. MS/MS of control RNA/DNA (upper); control + paromomycin (lower)

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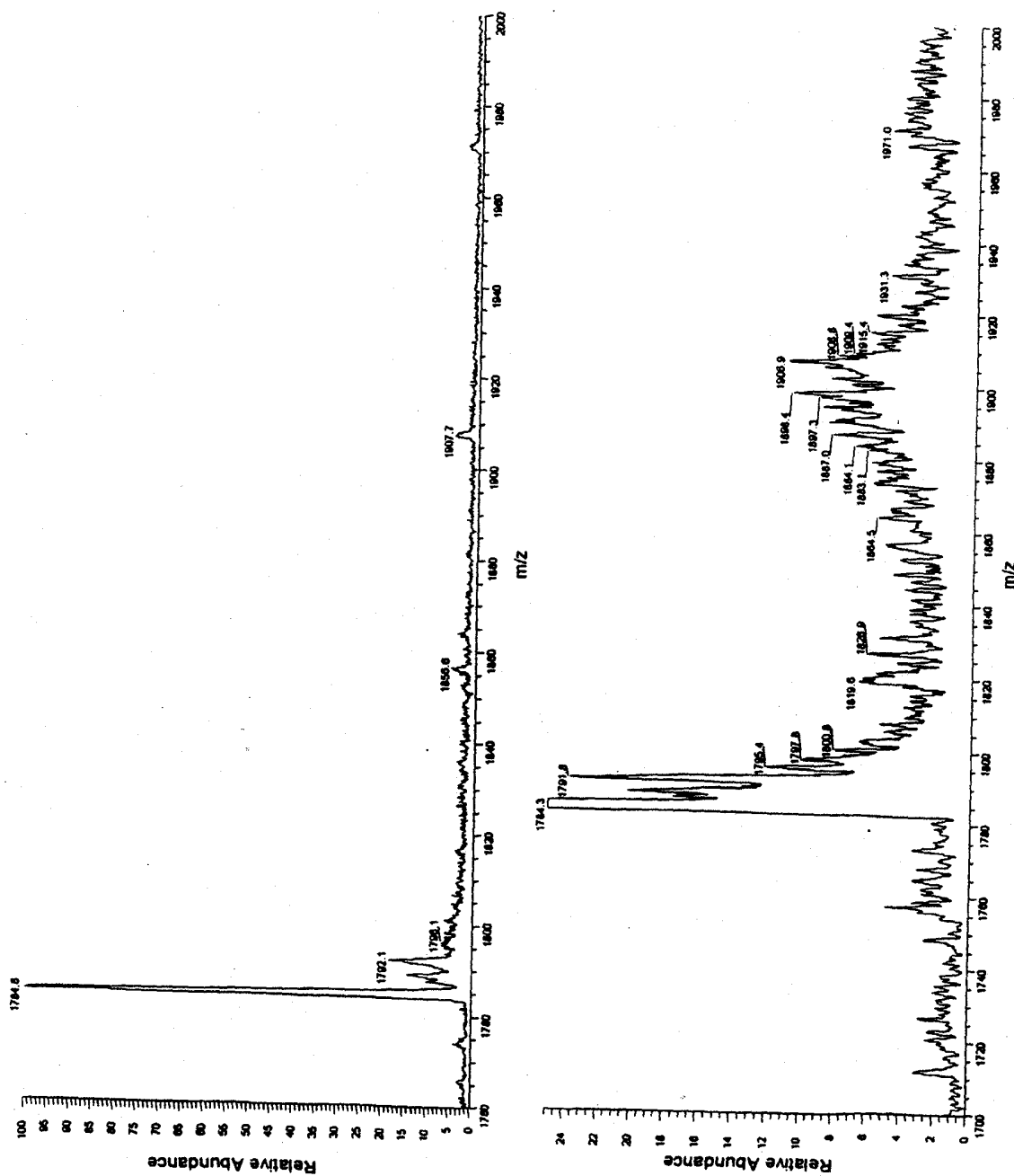


Figure 3. RNA/DNA chimera+paramomycin (upper); chimera+library (lower)

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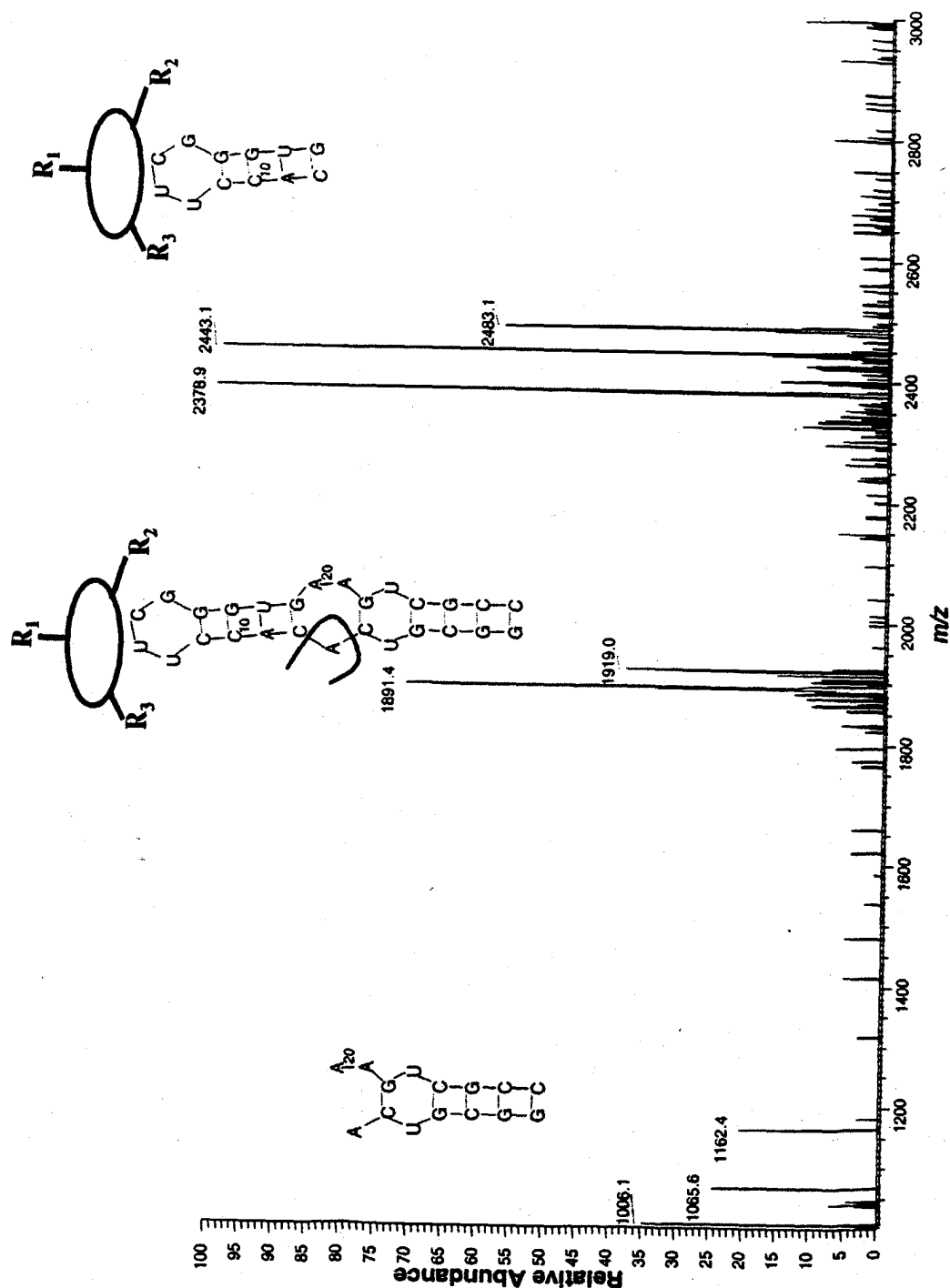


Figure 4. MS-MS analysis of member bound to RNA/DNA chimera

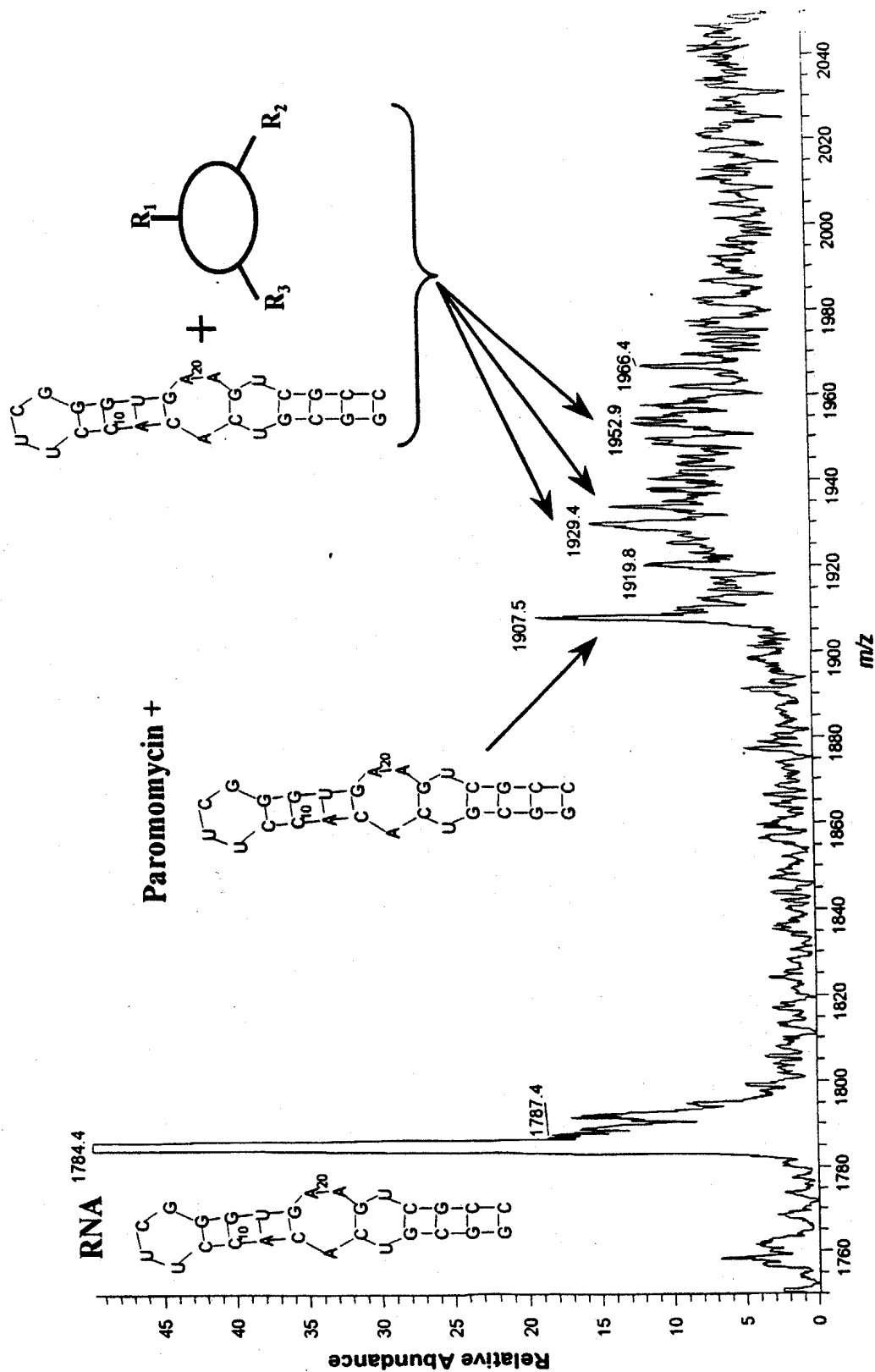


Figure 5. ESI-MS of RNA/DNA chimera bound to paromomycin and library

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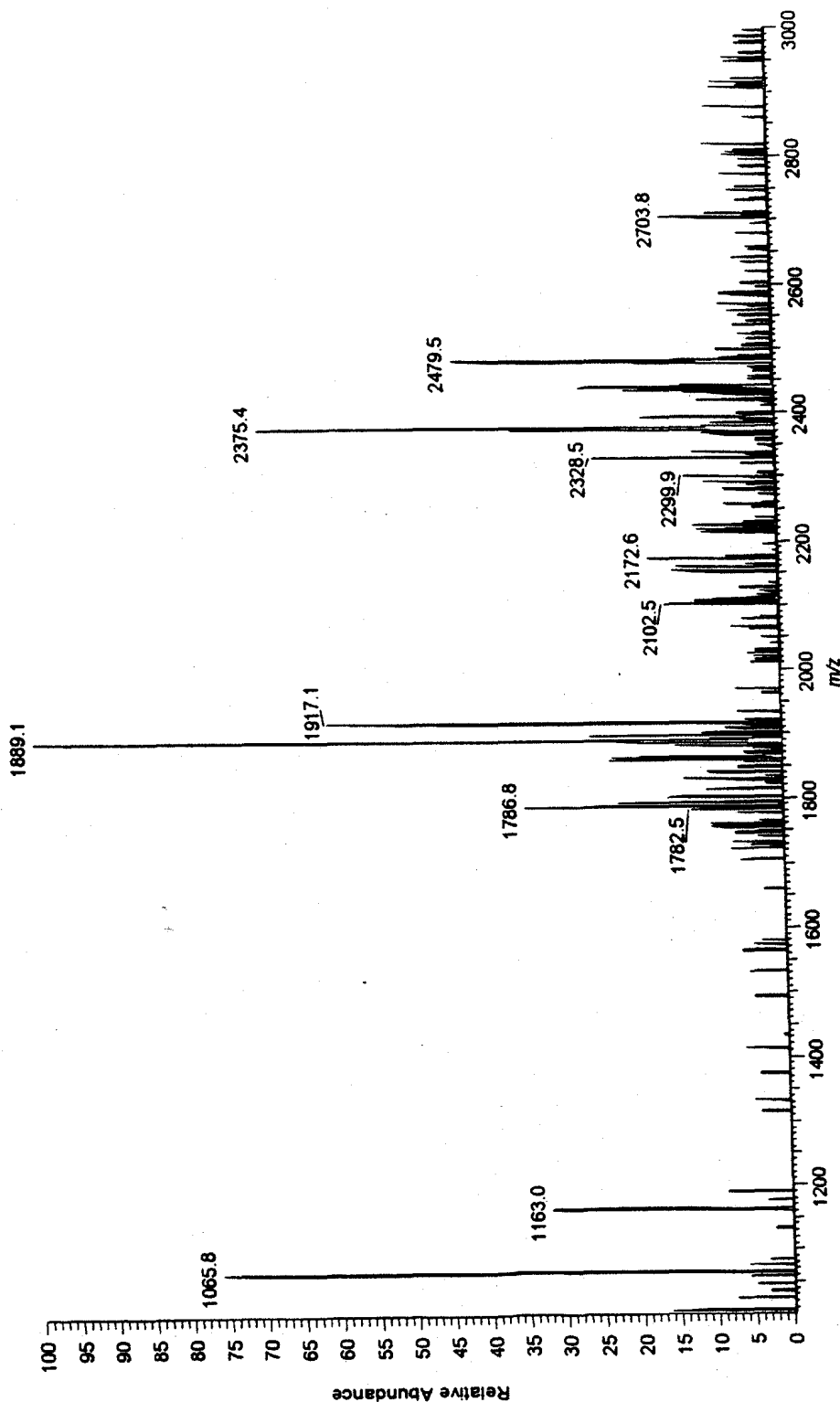


Figure 6. MS/MS of RNA/DNA chimera + compound with mass 665.1 not bound at the A-site

[illegible]

Figure 7. MS-MS analysis of member bound to RNA/DNA chimera at the A-Site

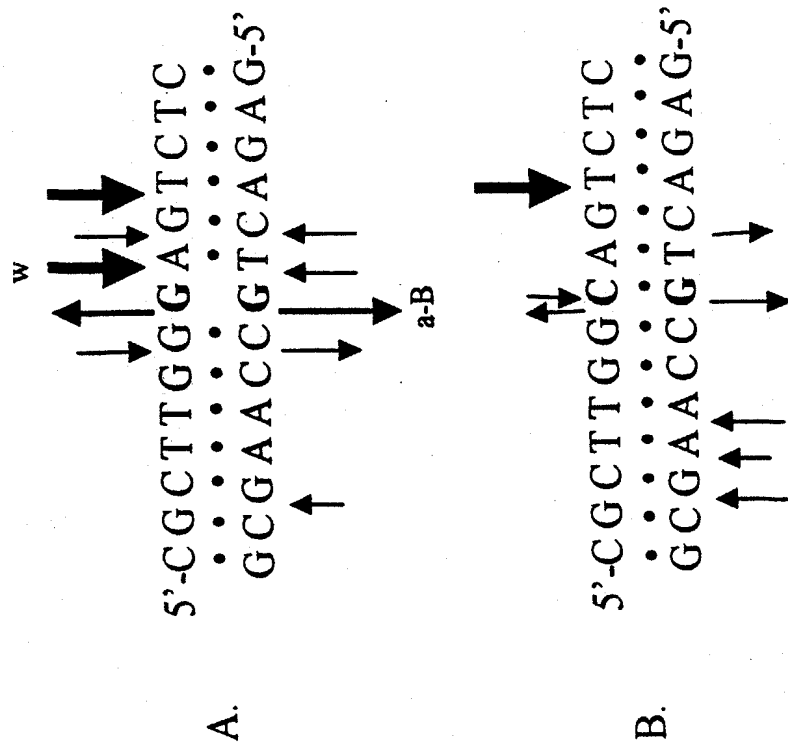


Figure 8. MS Fragmentation of DNA:DNA duplexes

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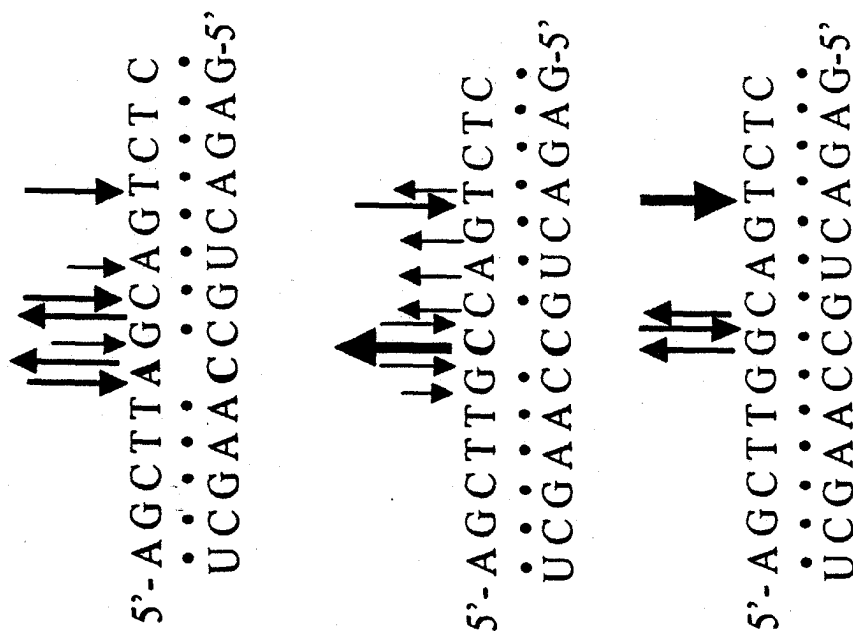


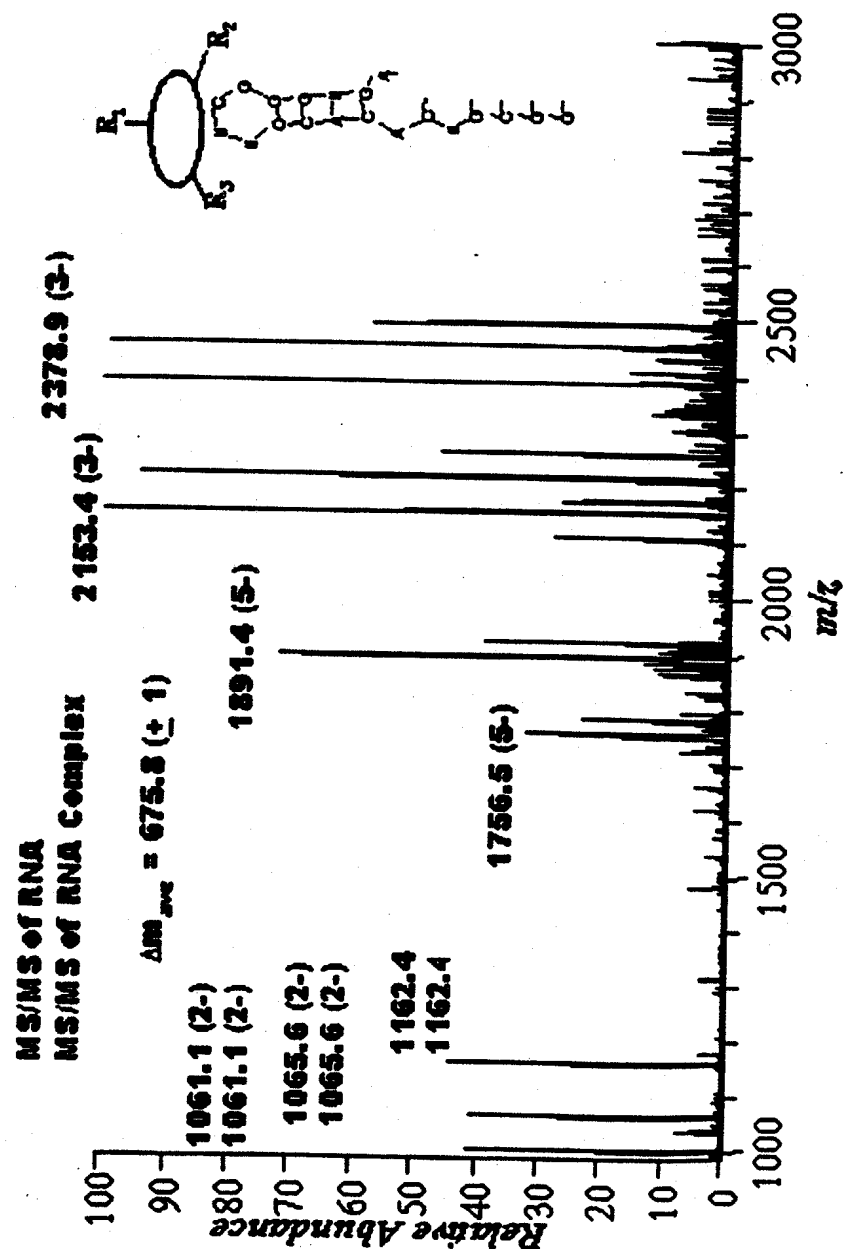
Figure 9. MS Fragmentation of DNA:RNA duplexes

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MASS Analysis of Binding Location

non-A site binder

FIGURE 10



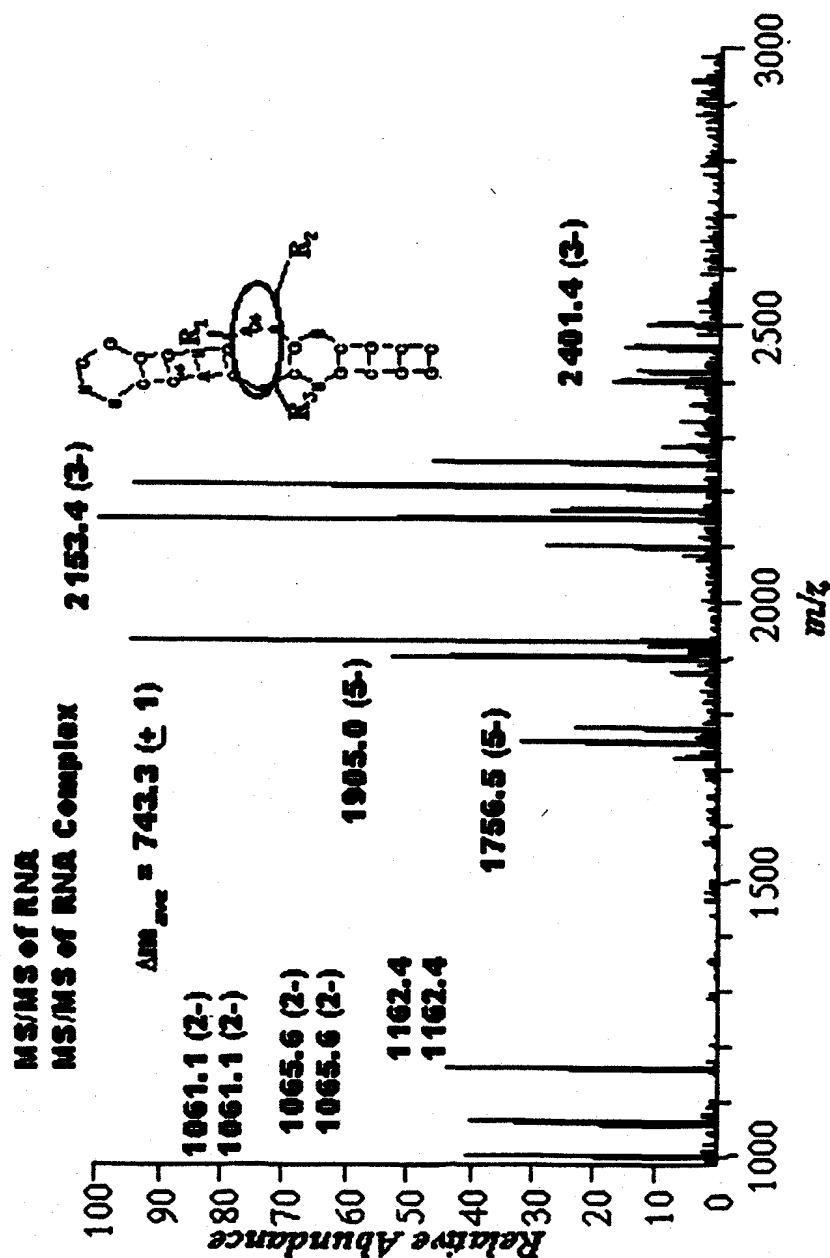
FO6T90* 47E+8860

MASS Analysis of Binding Location

non-A site binder

FIGURE 11

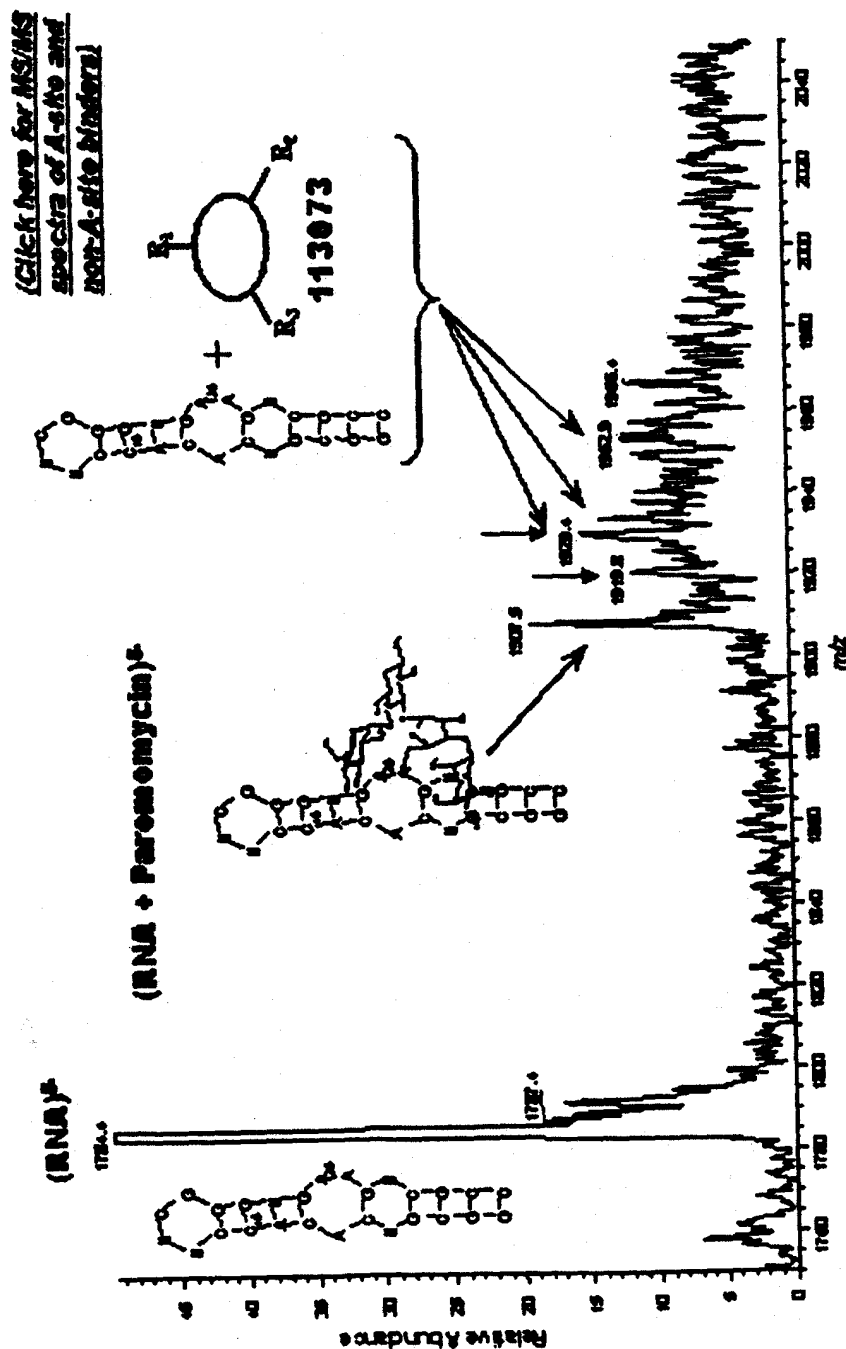
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MASS analysis of 16S A site RNA plus 216 member library (performed on quadrupole ion trap)

FIGURE 12



High Precision ESI-FTICR Mass Measurement of 16S A site RNA/Paromomycin Complex

Figure 13

use of unbound RNA as internal mass standard
 provides low ppm mass measurement errors

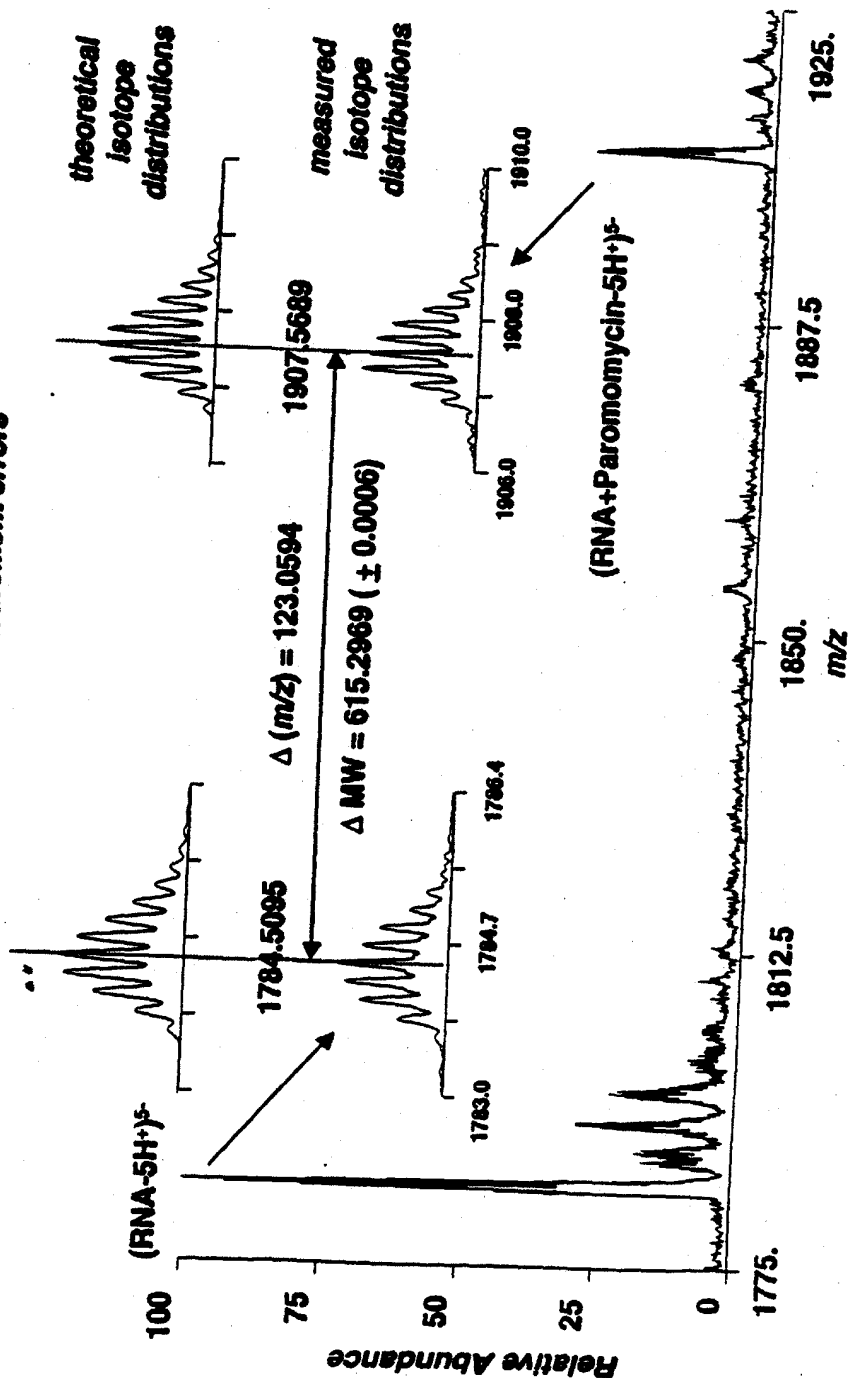
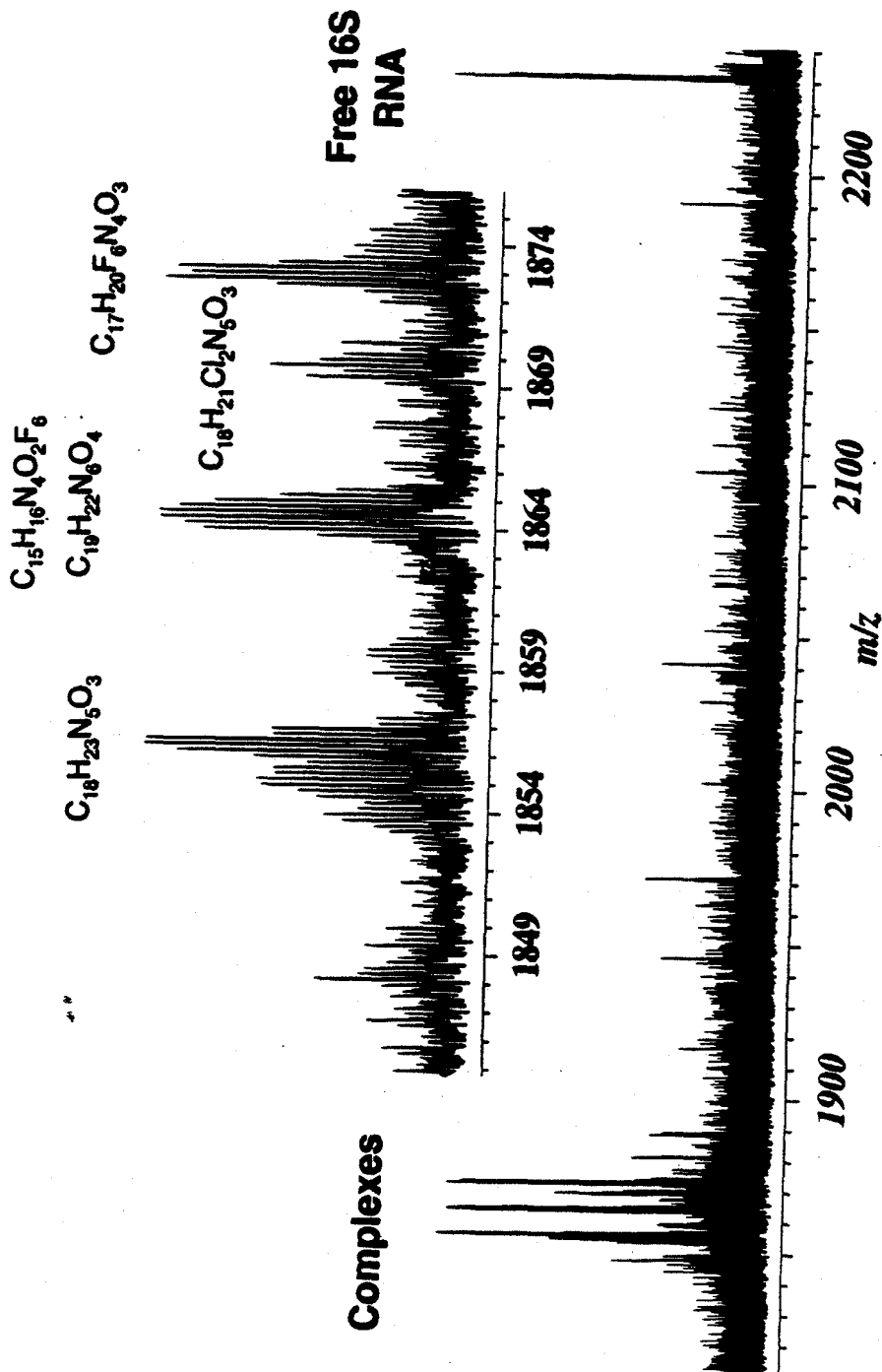


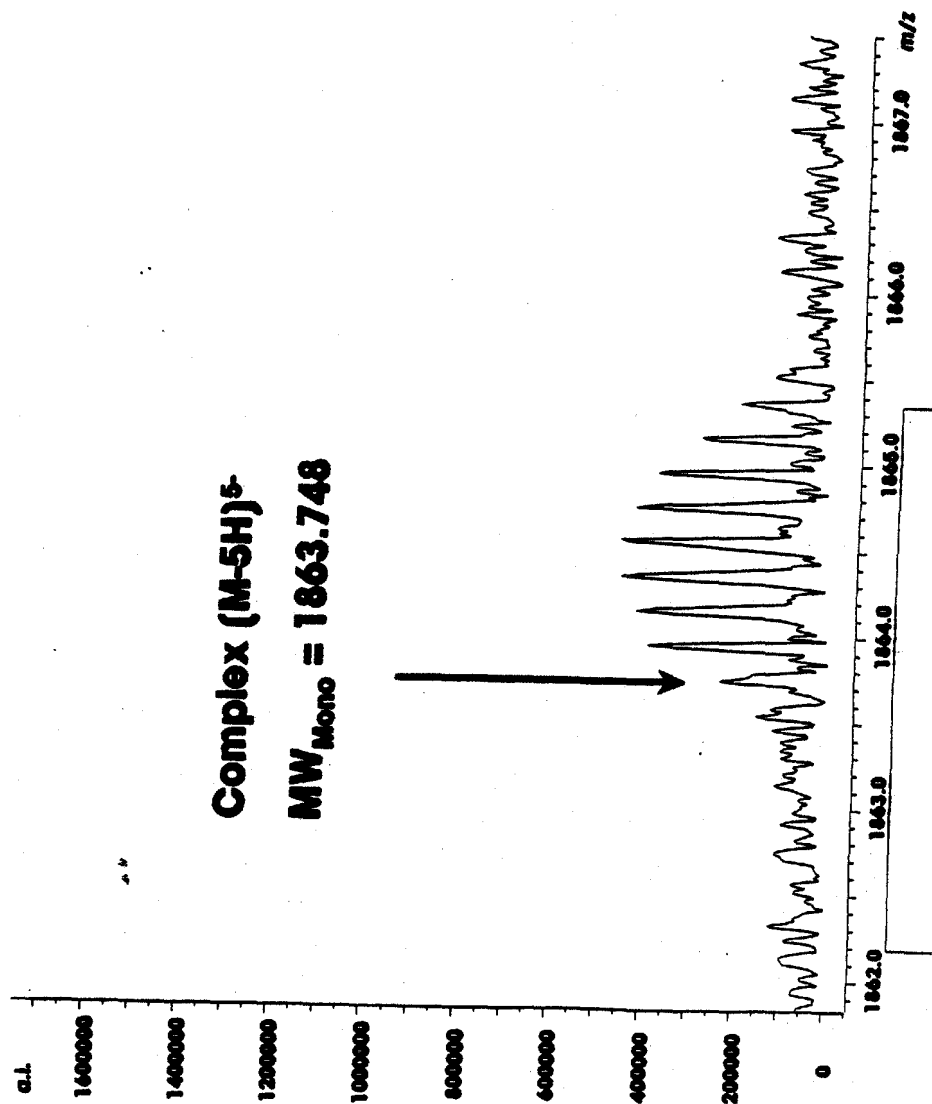
Figure 14
MASS of 60-Member Ibis Library Against 16S A-site RNA



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Figure 15
MASS of 60-member Library against 16S A-site Model



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Figure 16

FT-ICR MS of Starting Library

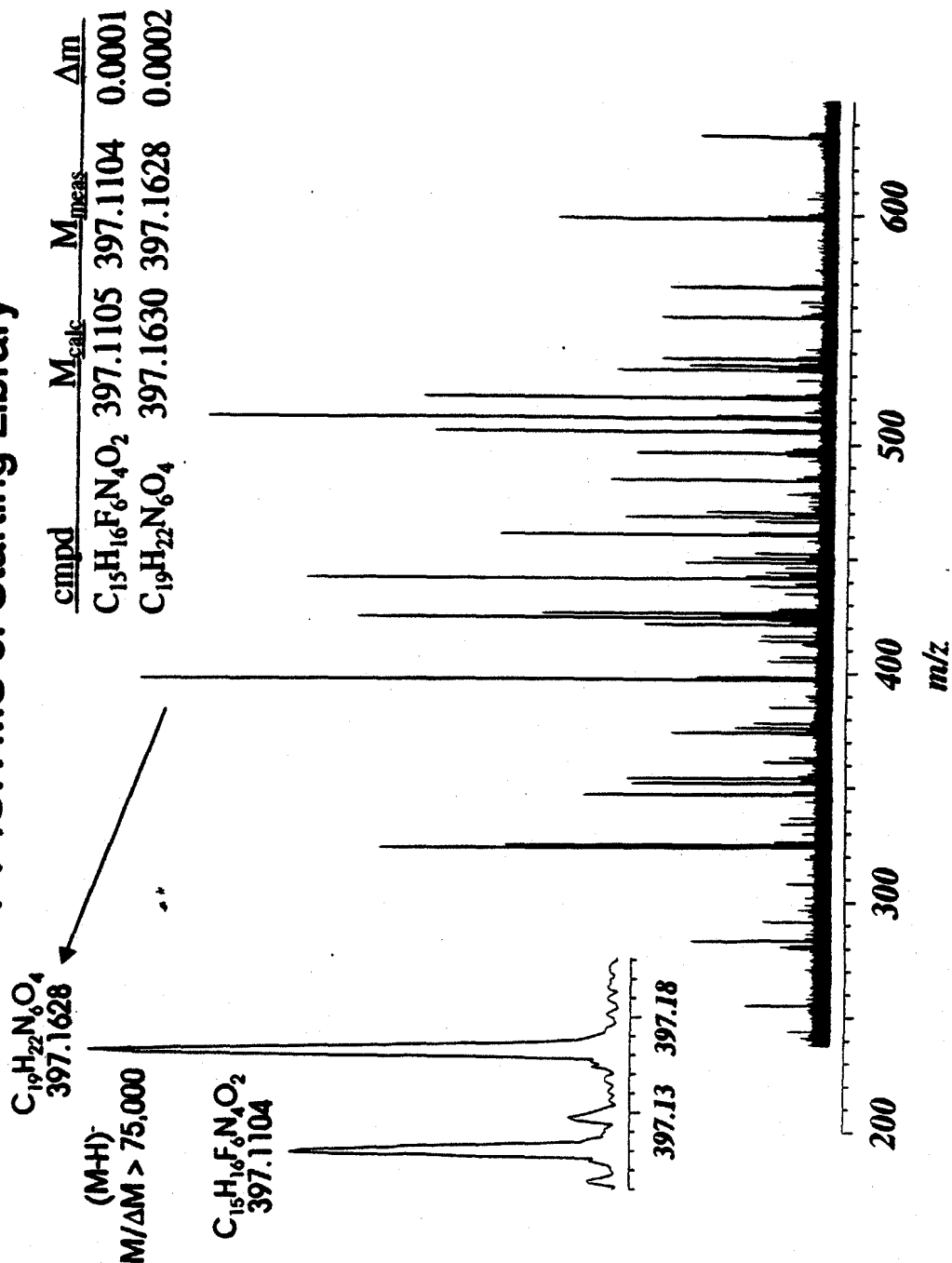


Figure 17
 Compound Identification from a 60-member
 Combinatorial library with MASS

Complex M_{meas}	9320.300 \pm .009 Da
RNA M_{meas}	8922.189 \pm .009
ΔM	398.111 \pm .009 Da

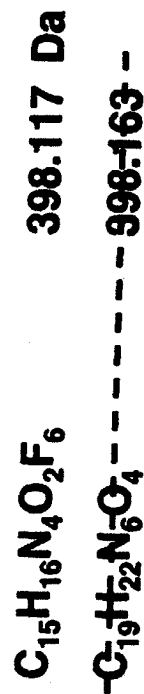


Figure 18

Elemental Composition Constraints

Measured Mass: 615.2969
 Mass Tolerance: 1.0 ppm
 Charge: 0

Element	Min. atoms	Max. atoms
^{12}C	12	30
^1H	23	60
^{16}O	7	20
^{14}N	3	20

Possible Elemental Compositions:

Calc. Mass (amu)	Error (ppm)	Molecular Formula
615.296291	0.98	$^{16}\text{O}_4$ $^{14}\text{N}_{19}$ $^{12}\text{C}_{21}$ $^1\text{H}_{33}$
615.296298	0.98	$^{16}\text{O}_9$ $^{14}\text{N}_{12}$ $^{12}\text{C}_{22}$ $^1\text{H}_{39}$
615.296305	0.97	$^{16}\text{O}_{14}$ $^{14}\text{N}_5$ $^{12}\text{C}_{23}$ $^1\text{H}_{45}$
615.296808	0.15	$^{16}\text{O}_{15}$ $^{14}\text{N}_{17}$ $^{12}\text{C}_8$ $^1\text{H}_{41}$
615.296815	0.14	$^{16}\text{O}_{20}$ $^{14}\text{N}_{10}$ $^{12}\text{C}_9$ $^1\text{H}_{47}$

Further constrain by
 elemental
 composition of
 "letters"
 unintended
 products...

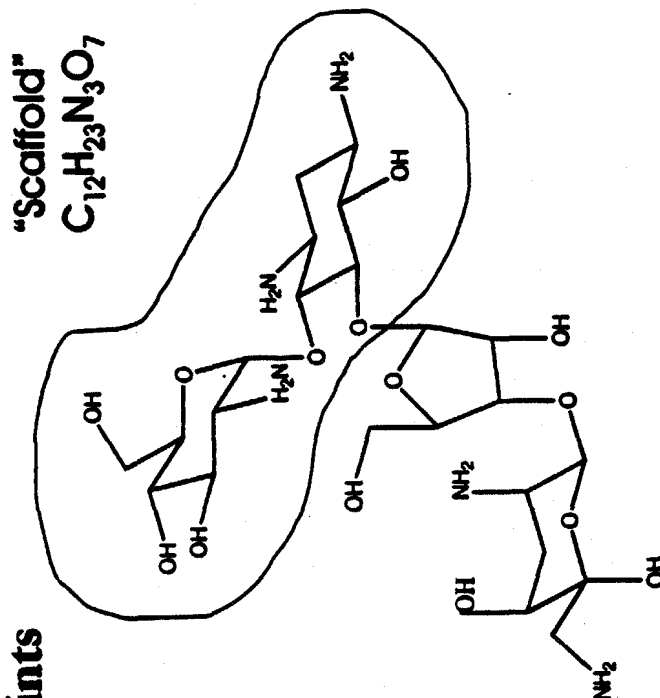
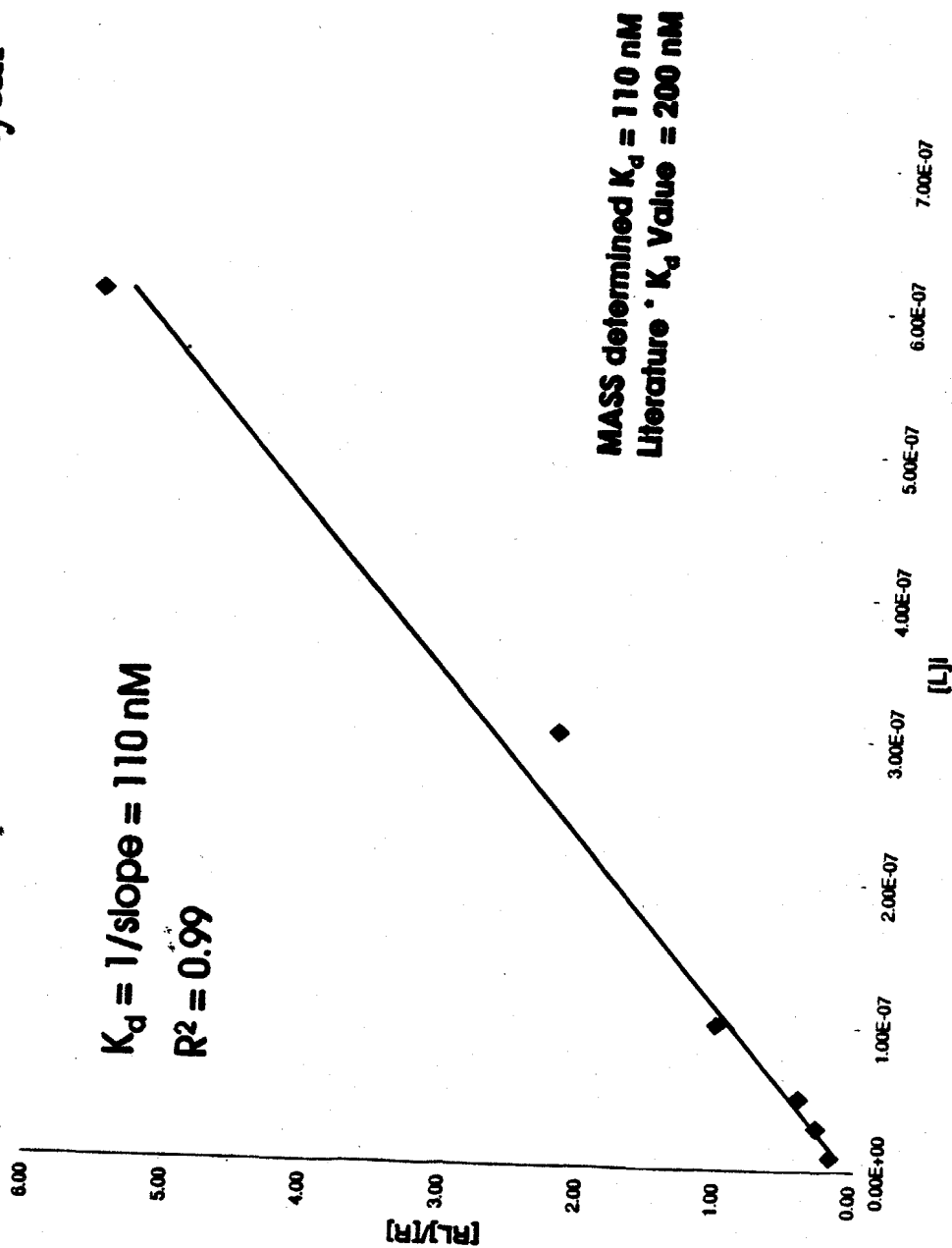


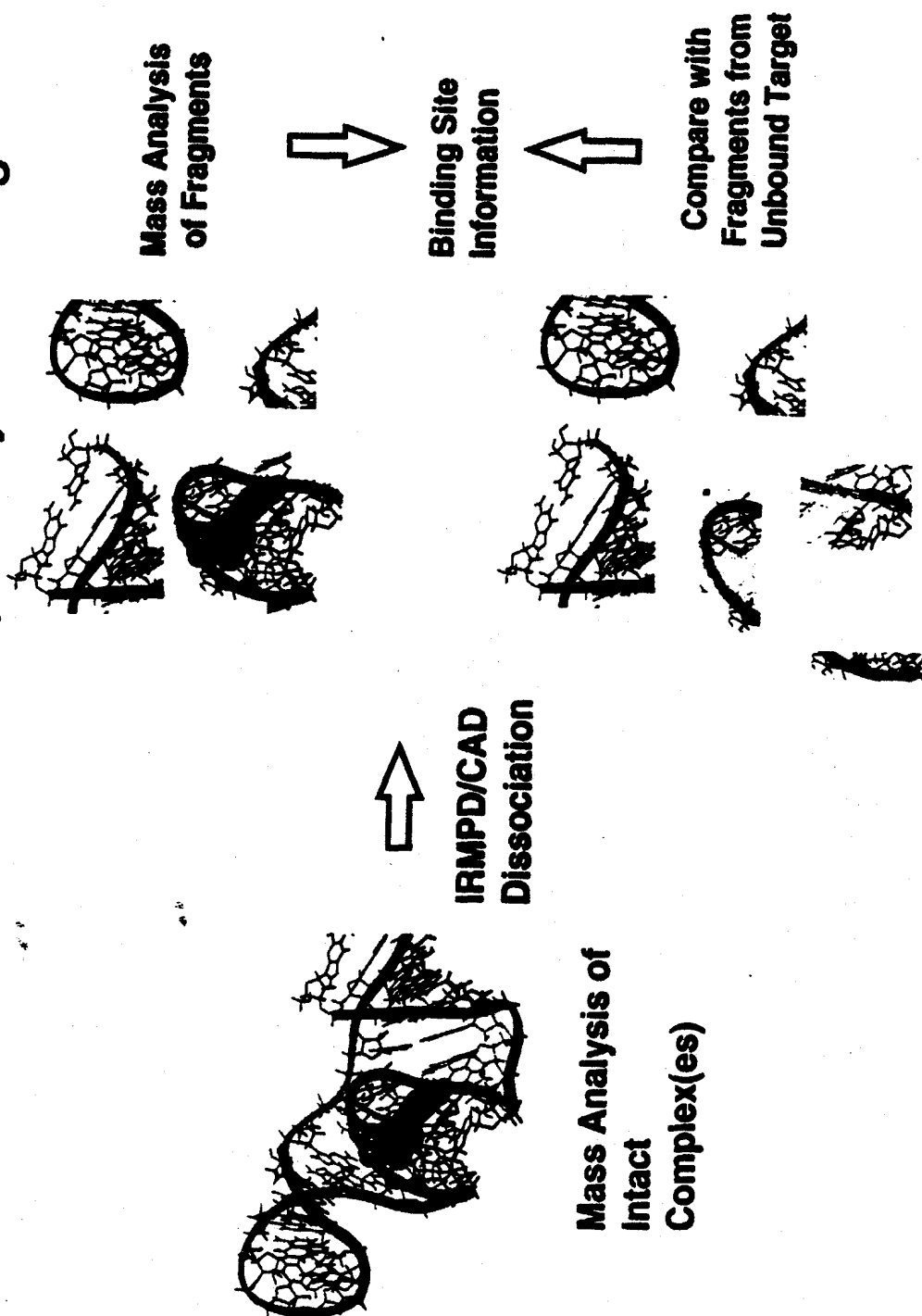
Figure 19
 MASS K_d determination for 16S-Paromomycin



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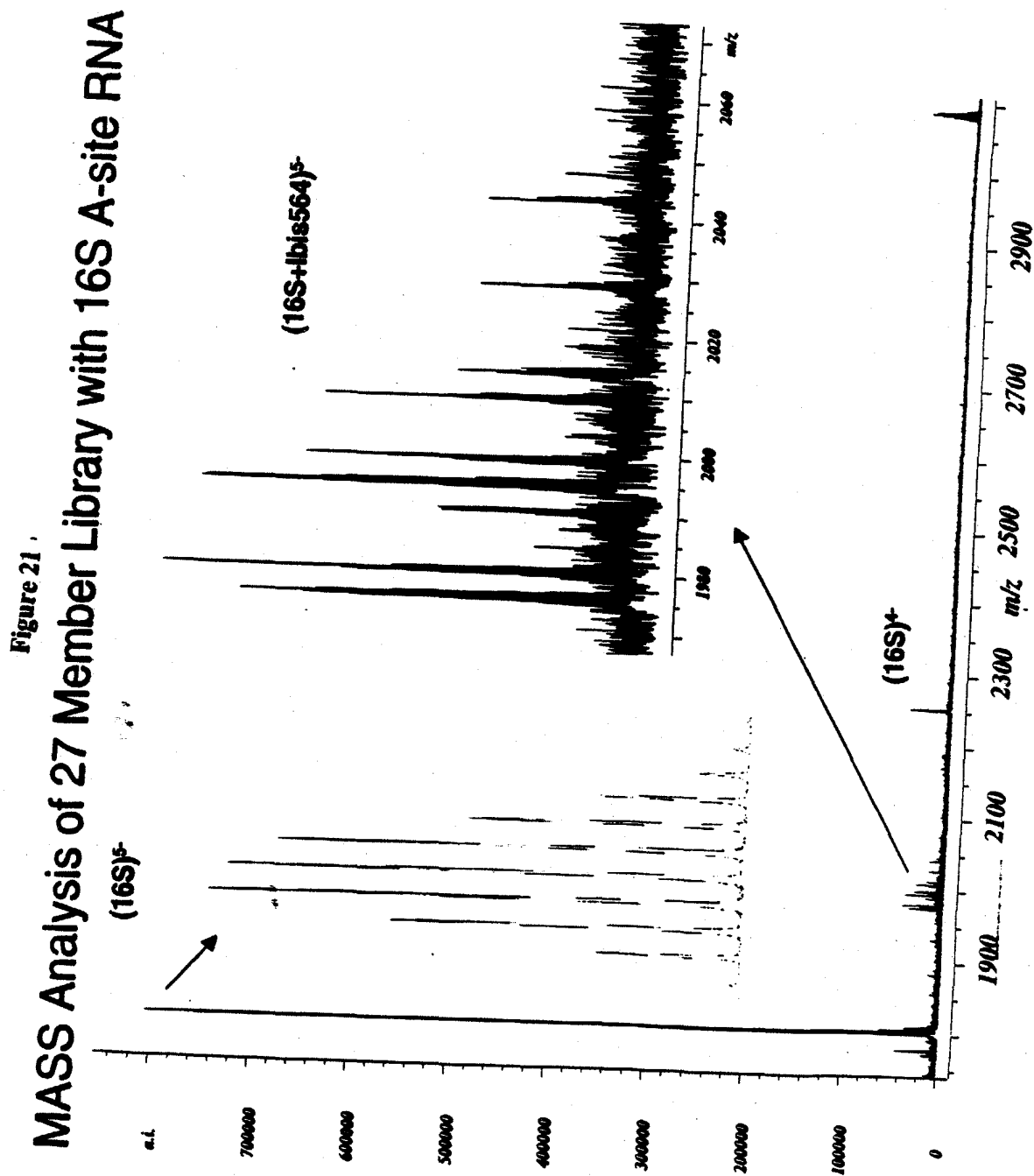
Multitarget Affinity/Specificity Screening

Figure 20



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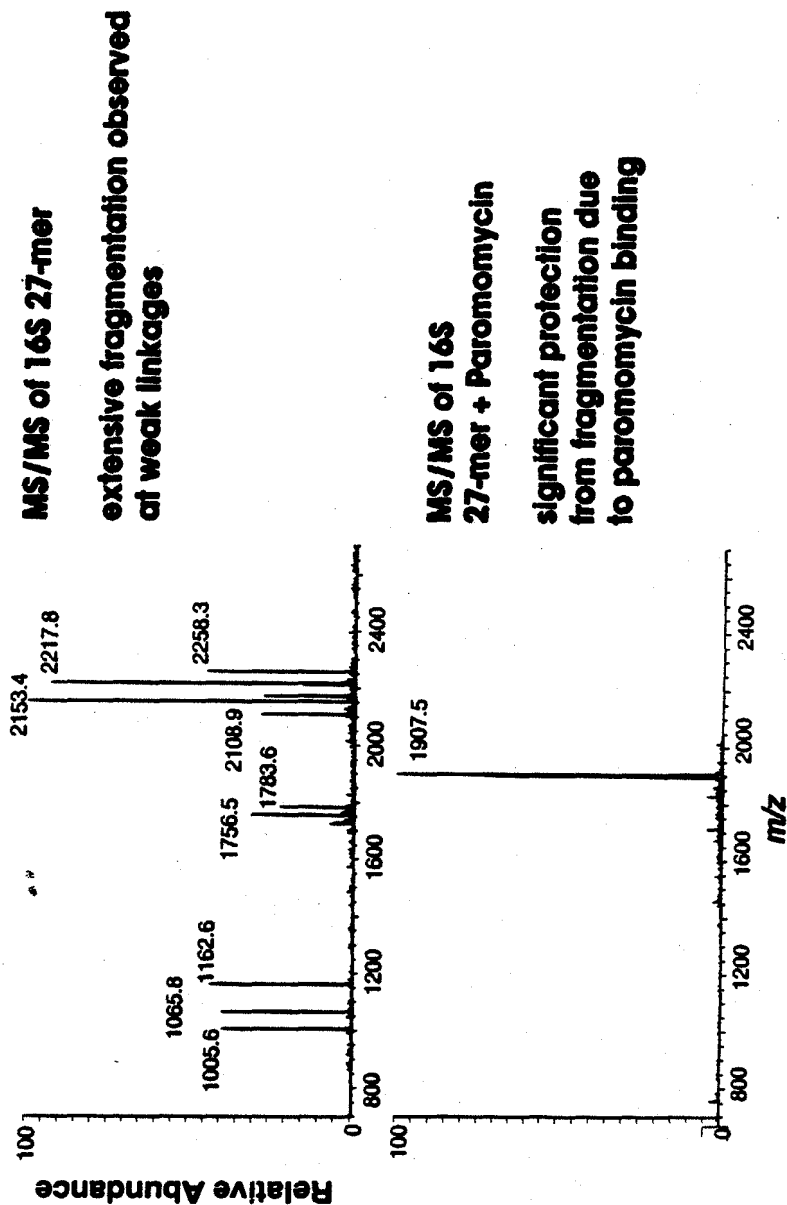


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Figure 22

MASS Protection Assay

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Figure 23

MASS Protection Assay

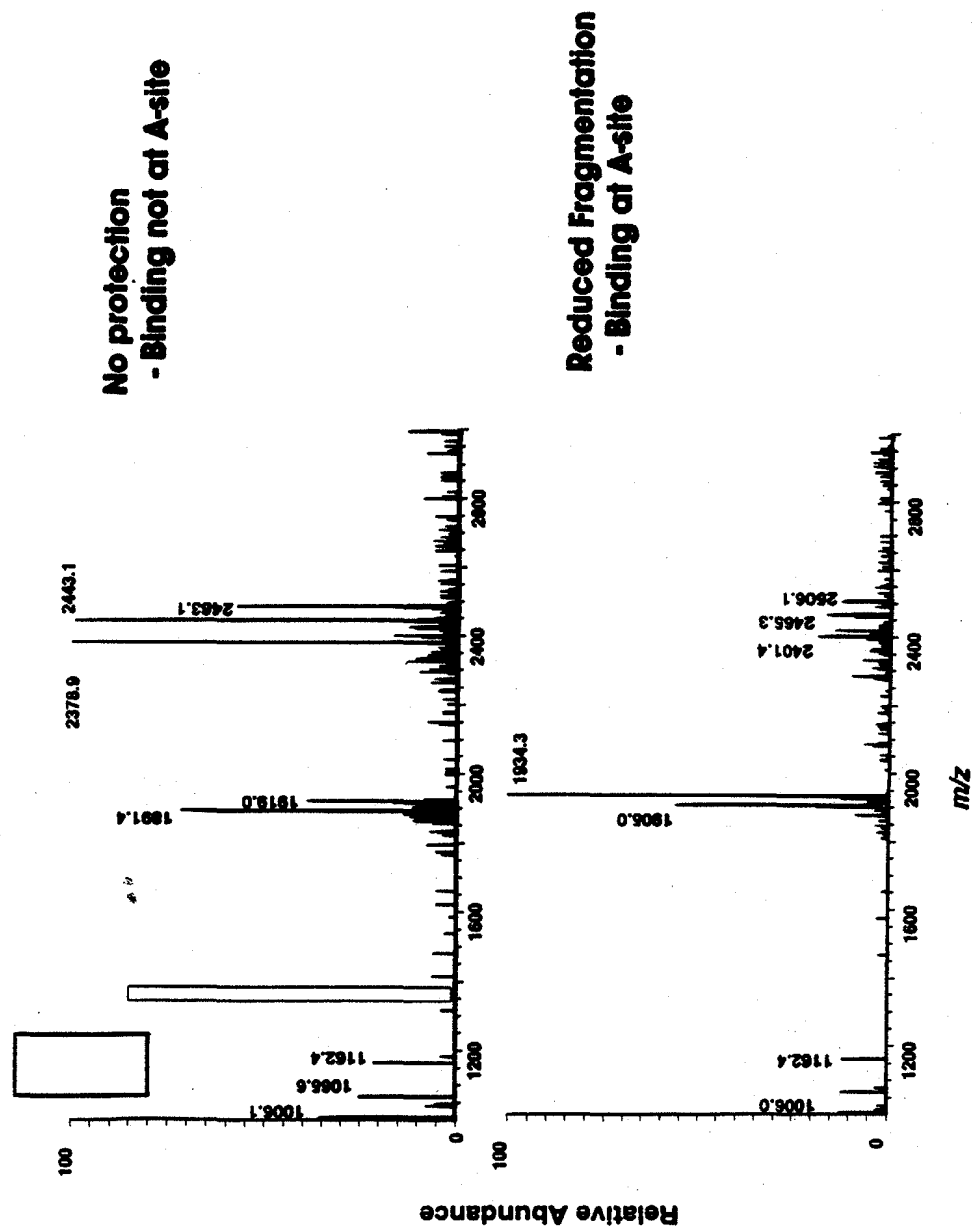
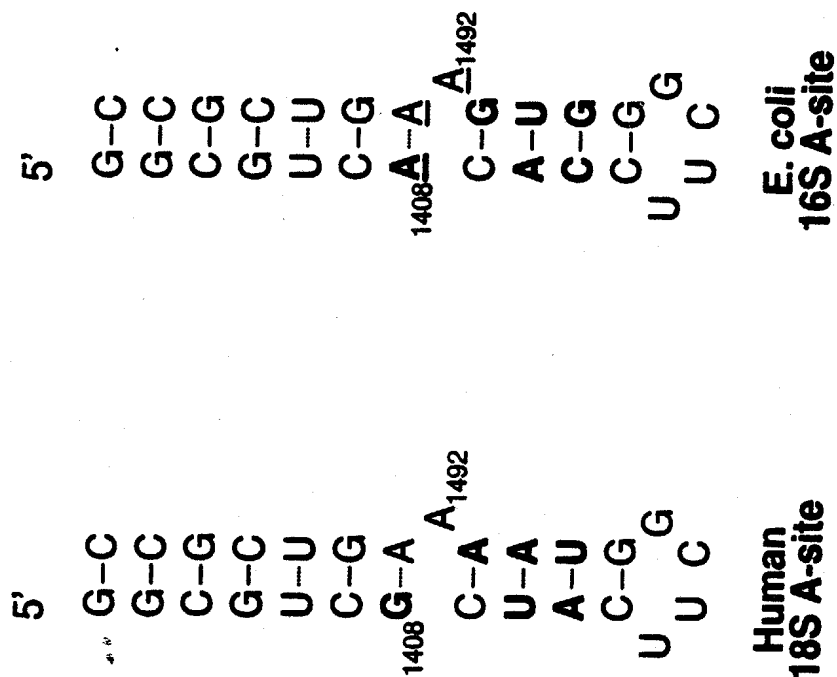


Figure 24
Eukaryotic and Prokaryotic A-Sites
 Aminoglycoside antibiotics bind to
 A-site of decoding region in 16S RNA



Δ MW = 15.011 Da

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Figure 25
Neutral Mass Tag Does Not Affect Ligand Binding

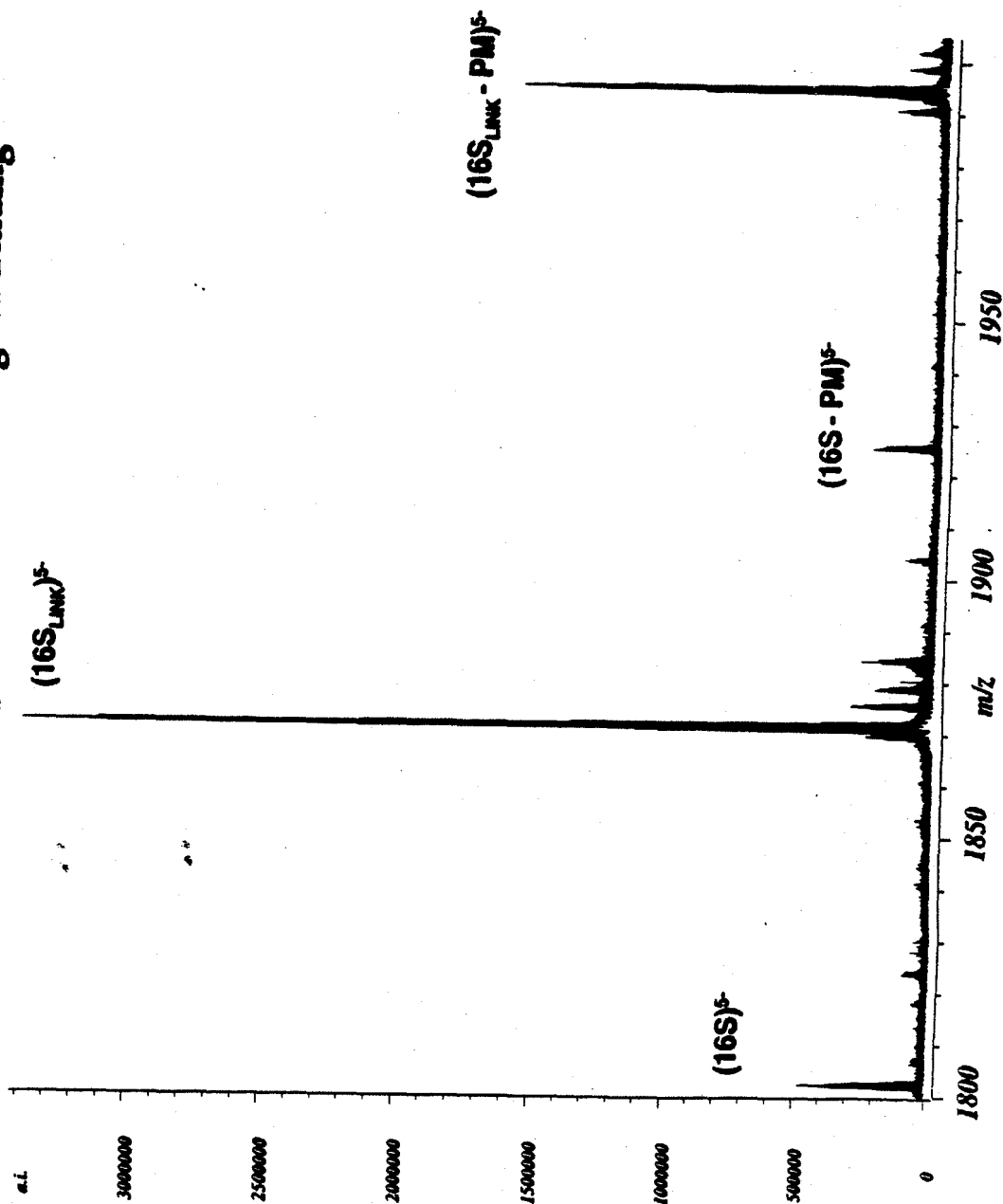


Figure 26

Simultaneous Screening of 16S A-site and 18S A-site Model RNAs Against Aminoglycoside Mixture

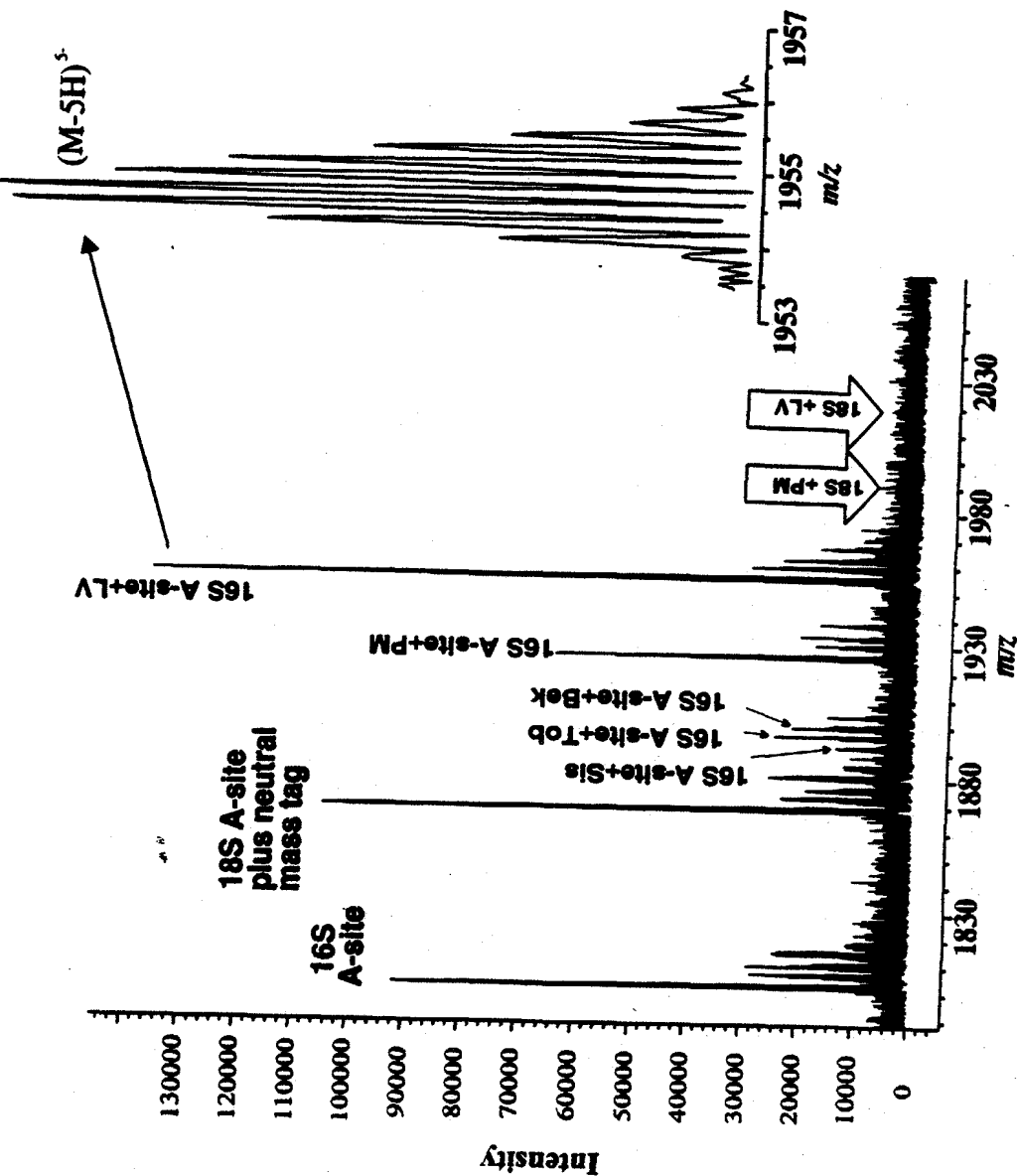
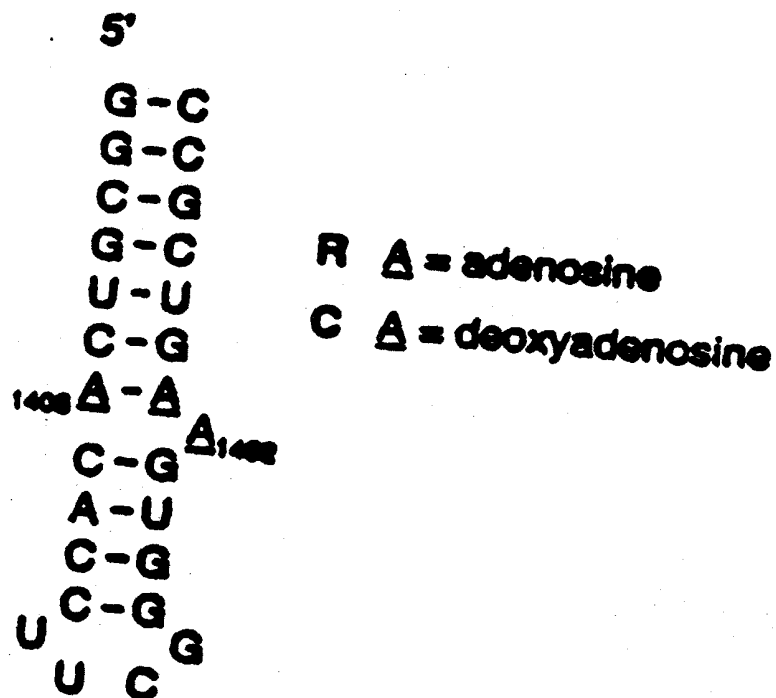


Figure 27



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Figure 28

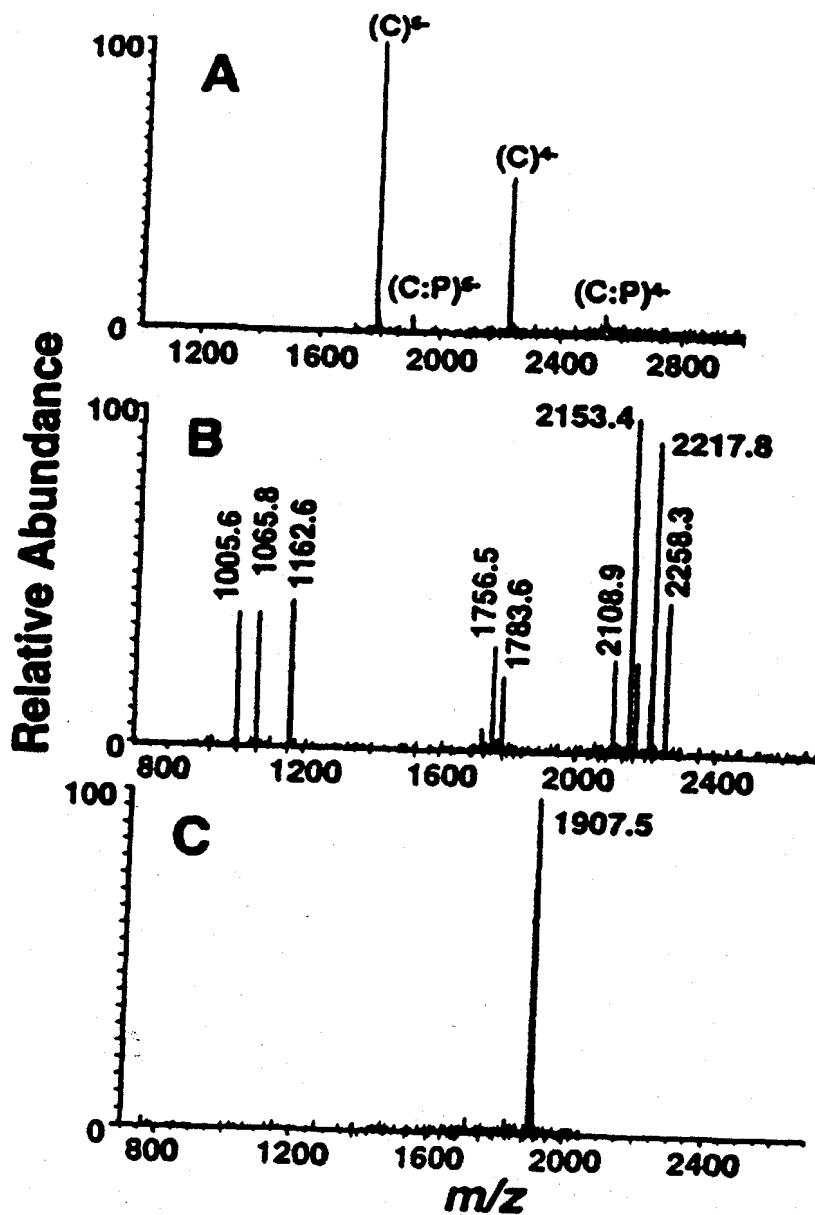
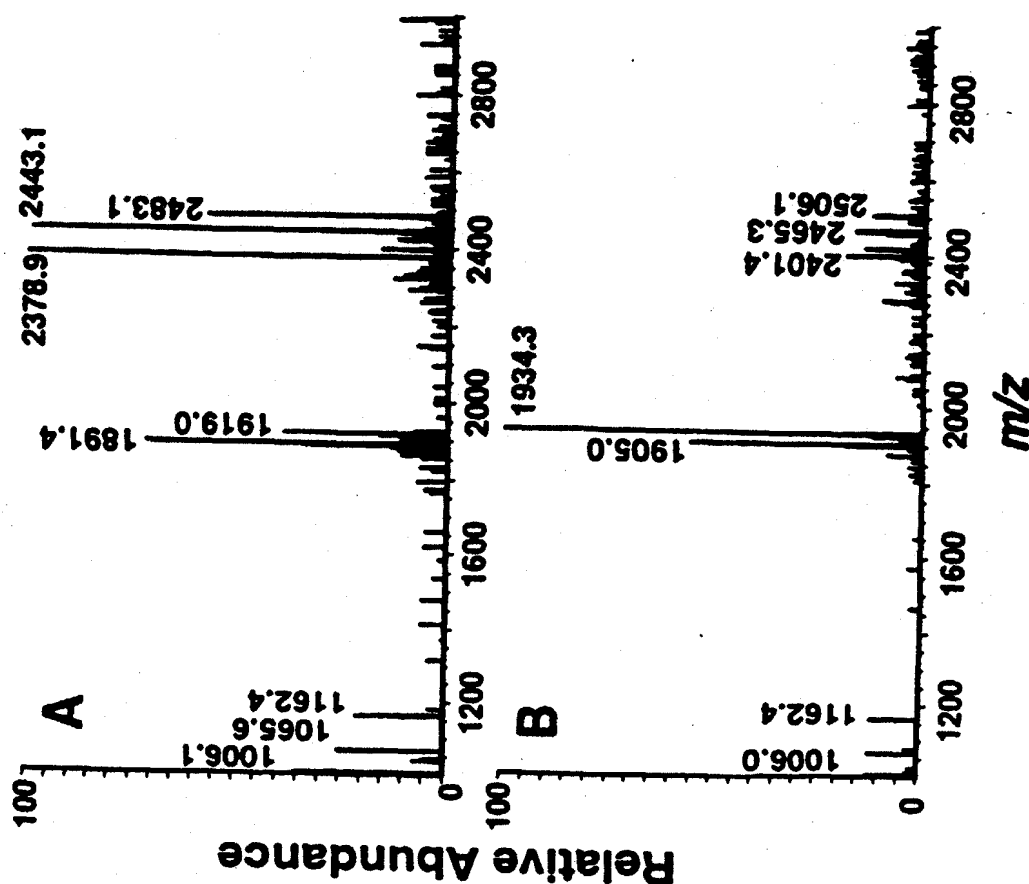


Figure 29



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Figure 30

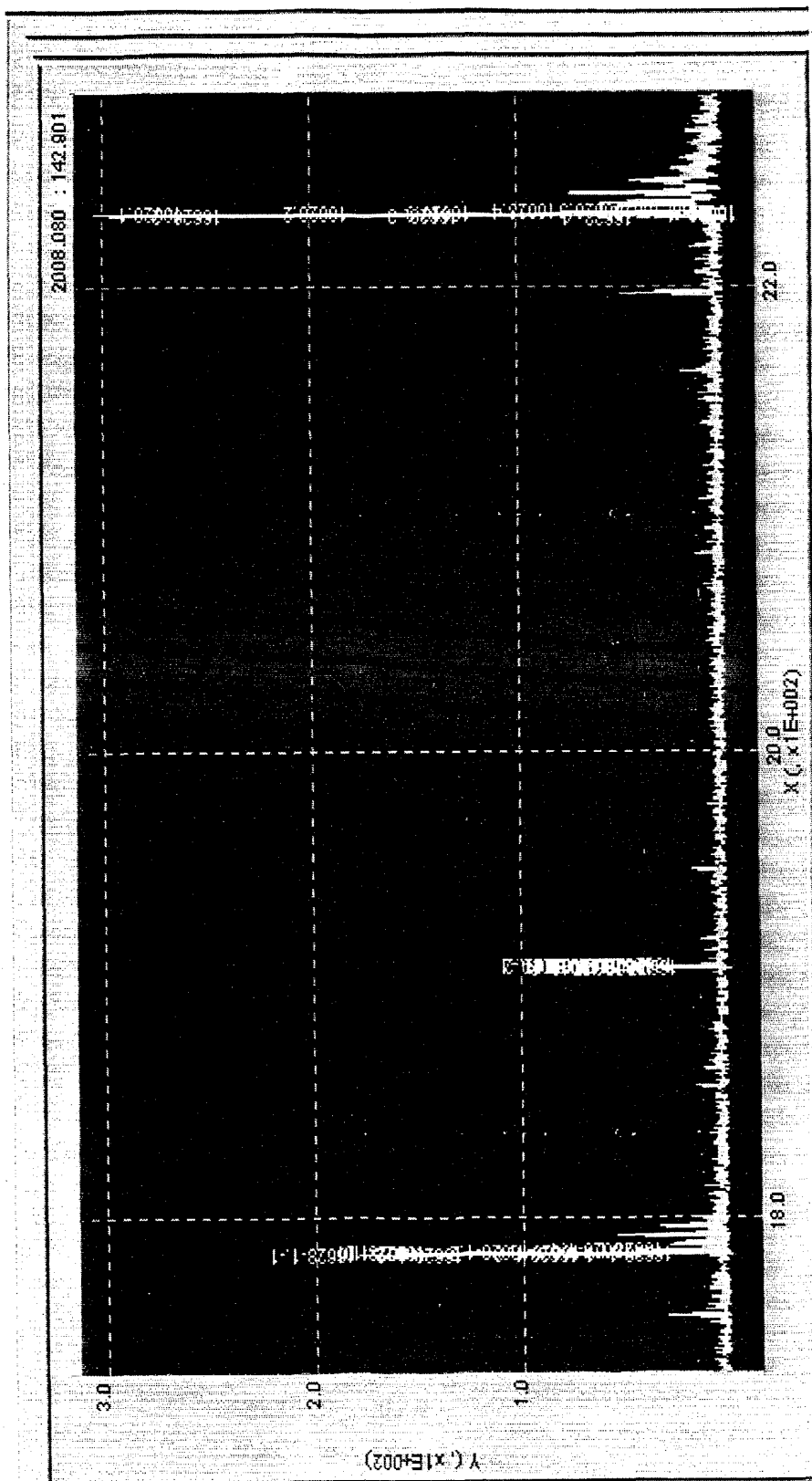
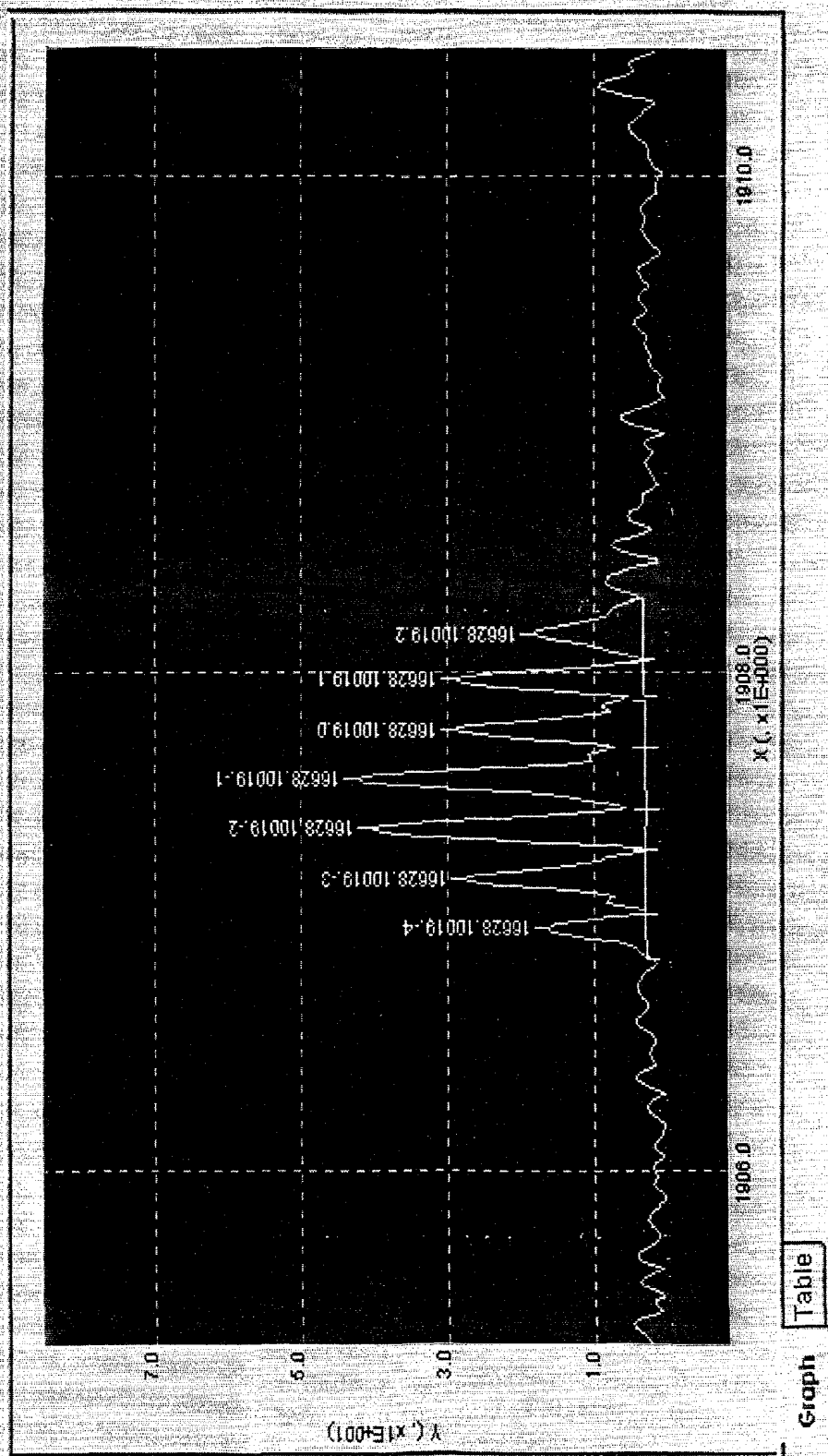


Figure 31



Graph Table

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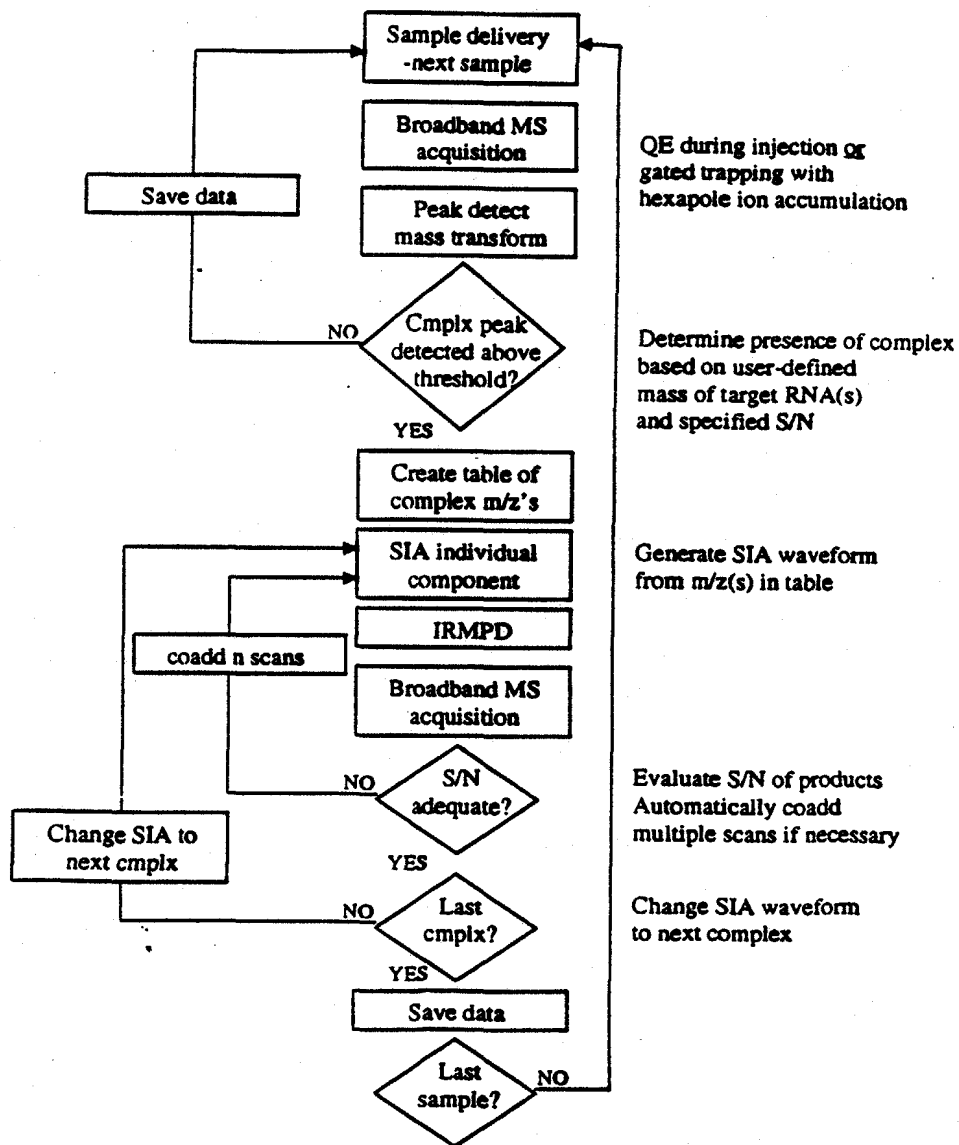
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Figure 32

nr	name	apex	start	stop	height	area
1	16628-1.4	1783.710	1783.635	1783.834	14.55	1.63
2	16628-1.3	1783.909	1783.834	1783.972	60.04	5.15
3	16628-1.2	1784.109	1784.021	1784.184	115.60	11.14
4	16628-1.1	1784.308	1784.233	1784.383	167.34	15.89
5	16628-1.0	1784.508	1784.433	1784.620	133.94	14.74
6	16628-1.1	1784.707	1784.620	1784.795	136.60	13.38
7	16628-1.2	1784.907	1784.795	1784.982	82.63	8.56
8	16628-1.3	1785.107	1785.032	1785.219	57.81	5.21
9	16628-1.4	1785.306	1785.232	1785.369	32.31	2.65
10	16628-1.5	1785.506	1785.456	1785.569	17.67	1.12
11	16628.10019.4	1906.974	1906.874	1907.031	12.63	1.00
12	16628.10019.3	1907.173	1907.045	1907.273	22.54	2.11
13	16628.10019.2	1907.373	1907.287	1907.444	33.86	2.91
14	16628.10019.1	1907.572	1907.458	1907.701	34.87	3.30
15	16628.10019.0	1907.772	1907.701	1907.843	20.93	1.55
16	16628.10019.1	1907.972	1907.900	1908.043	21.03	1.55
17	16628.10019.2	1908.157	1908.086	1908.271	10.97	0.90
18	16628.4	2229.874	2229.679	2230.029	27.51	4.87
19	16628.3	2230.146	2230.029	2230.263	111.72	16.23
20	16628.2	2230.380	2230.263	2230.516	225.18	32.39
21	16628.1	2230.633	2230.516	2230.770	280.66	40.90
22	16628.0	2230.887	2230.770	2231.023	287.24	41.95
23	16628.1	2231.140	2231.023	2231.257	242.23	34.17

Graph Table

Figure 33



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